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Rabies RT-PCR Detection Kit

Product Insert

Product # 45300

Pathogen Information

Rabies is a viral disease that results in acute encephalitis (inflammation of the brain) in mammals. Rabies is caused by the rabies virus, which is a non-enveloped virus with a single-stranded RNA genome. The virus is transmitted via saliva, and transmission of the disease almost always occurs as a result of an infected animal biting a non-infected animal. The virus does not survive long outside the host and will remain viable in the carcass of an infected animal for less than 24 hours. After coming into contact with the virus, rabies will spread through the nerves towards the brain. The virus is slow moving and the average time of incubation from exposure to brain involvement is between 3 to 8 weeks in a dog and 2 to 6 weeks in a cat. However, incubation periods as long as 6 months have been reported. After the virus reaches the brain, it will then spread to the salivary glands where it can spread through bites. The virus is shed at high levels in saliva. There is no treatment for the disease, once rabies develops it is fatal. Currently, the only way to diagnose rabies in animals is to submit the brain for post-mortem examinations. Since it is possible to be exposed to rabies without becoming infected, methods for the diagnosis of disease by other methods is urgently required. This will not only help in the prevention of the spread of disease, but may also spare animals that would otherwise be euthanized.

Principle of the Test

Norgen's Rabies RT-PCR Detection Kit constituents a ready-to-use system for the isolation and detection of rabies virus using end-point one-step RT-PCR. The kit first allows for the isolation of rabies RNA from the blood samples using spin-column chromatography based on Norgen's proprietary resin. The RNA is isolated free from inhibitors, and can then be used as the template in a one step RT-PCR reaction for rabies detection using the provided Rabies Detection Mastermix. The Rabies Detection Mastermix contains reagents and enzymes for the specific amplification of a 280 bp region of the viral genome. In addition, Norgen's Rabies RT-PCR Detection Kit contains a second Mastermix, the RT-PCR Control Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate RT-PCR reaction with the use of the provided *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. This kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Lysis Solution	10 mL
Wash Solution	11 mL
Elution Buffer	2 mL
Mini Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
Rabies 2x RT-PCR Mastermix	0.35 mL
Control 2x RT-PCR Mastermix	0.35 mL
Isolation Control (IsoC)*[a]	0.3 mL
Rabies Positive Control (PosC)*[b]	0.1 mL
Nuclease Free-Water	1.25 mL
Norgen's DNA Marker	0.1 mL
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** IsoC = Isolation Control ; PosC= Positive Control*
[a] The isolation control is an RNA transcript.
[b] The positive control is Rabies RNA transcript

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 95-100% ethanol
- Thermocycler and or Real-Time PCR System
- Micropipettes with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
- Laminar flow hood for extractions
- Vortex
- Sterile, nuclease-free aerosol-barrier micropipettor tips
- Microcentrifuge tube rack
- Disposable latex gloves
- β-mercaptoethanol

Storage Conditions and Product Stability

- The Rabies Positive Control and Rabies Isolation Control should be stored at -70°C. If needed, make aliquots of the controls according to the volume used in the protocol (10 µL of **Rabies *PosC*** or 20 µL of ***IsoC***) prior to freezing.
- The **Rabies 2X RT-PCR Mastermix** and the **Control 2X RT-PCR Mastermix** should be stored at -20°C upon receipt (-70°C for long-term). Make appropriate aliquots and store at -20°C if needed.
- All other kit components may be stored at room temperature
- The **Rabies 2X RT-PCR Mastermix** and the **Control 2X RT-PCR Mastermix**, Positive Control and Isolation Control should not undergo repeated freeze-thaw (a maximum freeze-thaw of three times).
- For RT-PCR:
    - Allow reagents to thaw at room temperature prior to use
    - When thawed, mix the components and centrifuge briefly
    - Work quickly on ice.
    - After addition of RT-PCR Mastermix use within one hour

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Rabies RT-PCR Detection Kit, including the Rabies 2x RT-PCR Mastermix, Control 2X RT-PCR Mastermix, Isolation Control and Rabies Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Rabies RT-PCR Detection Kit is designed for research purposes only.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Disclaimers

The **Lysis Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Safety Information
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

General Precautions

- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
- Dispose of unused kit reagents and specimens according to local, provincial or federal regulations.
- Workflow in the laboratory should proceed in a uni-directional manner, beginning in the pre-amplification area(s) (i.e. specimen collection and RNA extraction) and moving to the amplification / detection area(s) (RT-PCR and gel electrophoresis).
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.

Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only.
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water.
- Clean all surfaces with commercially available RNase decontamination solutions.
- When working with purified RNA samples, ensure that they remain on ice during downstream applications.

INSTRUCTIONS FOR USE

Important Notes Prior to Beginning Protocol:

- Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.
- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed microcentrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution** by adding 25 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 36 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- It is important to work quickly during this procedure.
- **Isolation Control (*IsoC*)**
    - An Isolation Control (***IsoC***) is supplied. This allows the user to control the RNA isolation procedure. For this assay, add the Isolation Control *(**IsoC**)* to the lysate during the isolation procedure
    - The Isolation Control *(**IsoC**)* must not be added to the sample material directly.
    - Do not freeze and thaw the Isolation Control *(**IsoC**)* more than 2 times.
    - The Isolation Control *(**IsoC**)* must be kept on ice at all times during the isolation procedure.
- The RT-PCR components of the Rabies RT-PCR Detection Kit should remain at -20°C until RNA is extracted and ready for RT-PCR amplification.
- It is recommended that no more than 100 µL of blood be used in order to prevent clogging of the column.
- We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA or heparin as the anti-coagulant.
- Rabies has a poor survival rate outside the infected body. It is important to add the **Lysis Solution** to the specimen as soon as possible (within 6 hours)
- It is important to work quickly during this procedure.

A. SPECIMEN LYSATE PREPARATION

Blood Lysate Preparation:

1. Add 350 µL of the **Lysis Solution** to an RNase-free microcentrifuge tube.
2. Add up to 100 µL of blood. Vortex for 10 seconds to mix.

   Note: Rabies has a poor survival rate outside the infected body. It is important to add the **Lysis Solution** to the specimen as soon as possible (within 6 hours). In the presence of the Lysis Solution components, the virus could be stable for hours if stored at room temperature and > 1 month if stored at -70°C.

3. Add 10 µL of the Isolation Control (***IsoC***) to the lysate. Vortex for 10 seconds to mix.
4. Add 250 µL of 95% ethanol to the lysate. Vortex for 10 seconds to mix.
5. Proceed to RNA Isolation (Step B).

B. SPECIMEN RNA PURIFICATION

Following the lysate preparation viral RNA can be extracted from the specimens using the supplied buffers and solutions according to the following protocol:

1. Assemble a column with one of the provided collection tubes.
2. Apply the lysate with ethanol (up to 650 µL) to the column and centrifuge for 1 minute at 14,000 rpm.

   Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed through, spin for an additional minute.

3. Discard the flowthrough and reassemble the spin column with its collection tube.
4. Depending on lysate volume, repeat steps **B2** and **B3**.
5. Apply 400 µL of **Wash solution** and centrifuge for one minute at 14,000 rpm.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed through, spin for an additional minute.

6. Discard the flowthrough and reassemble the spin column with its collection tube.
7. Repeat steps **B5** and **B6** two more times (for a total of 3 washes).
8. Spin the column for 2 minutes to thoroughly dry the resin at 14,000 rpm. Discard the collection tube.
9. Place the column into a new 1.7 mL Elution tube.
10. Add 50 µL of **Elution Solution** to the column.
11. Centrifuge for 2 minutes at 2,000 rpm followed by a 2 minute spin at 14,000 rpm. Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column for an additional minute at 14,000 rpm.
12. The purified RNA sample could be used immediately for RT-PCR as described below. It is recommended that samples be placed at -70°C for long term storage.

C. Rabies RT-PCR Assay Preparation

Notes:

- Before use, suitable amounts of all RT-PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of **Rabies 2X RT-PCR Mastermix** and **Control 2X RT-PCR Mastermix** provided is enough for up to 32 RT-PCR reactions (24 sample RT-PCR, 4 positive control RT-PCR and 4 no template control RT-PCR) each.
- For each sample, one RT-PCR reaction using the **Rabies 2X RT-PCR Mastermix** and one RT-PCR reaction using **Control 2X RT-PCR Mastermix** should be set up in order to have a proper interpretation of the result.
- For every RT-PCR run, one reaction containing Rabies Positive Control (**Rabies *PosC***) and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of RNA samples tested per RT-PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of Rabies Limit of Detection.

1. Prepare the RT-PCR reaction for sample detection (Set #1, using **Rabies 2X RT-PCR Mastermix**) and the RT-PCR reaction for control detection (Set #2, using **Control 2X RT-PCR Mastermix**) as shown in Table 1 below. The recommended amount of sample RNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample RNA may be used as template. Ensure that one Rabies detection reaction and one control reaction is prepared for each RNA sample. Adjust the final volume of the RT-PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. RT-PCR Assay Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
Rabies 2X RT-PCR Mastermix Or Control 2X RT-PCR Mastermix	**10 µL**
Sample RNA	**2.5 µL**
Nuclease-Free Water	**7.5 µL**
Total Volume	**20 µL**

2. For each RT-PCR run, prepare **one** positive control RT-PCR as shown in Table 2 below:

Table 2. RT-PCR Positive Control Preparation

RT-PCR Components	Volume Per RT- PCR Reaction
Rabies 2X RT-PCR Mastermix Or Control 2X RT-PCR Mastermix	10 µL
Rabies *Positive Control (PosC)*	10 µL
Total Volume	20 µL

3. For each RT-PCR run, prepare **one** no template control RT-PCR as shown in Table 3 below:

Table 3. RT-PCR Negative Control Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
Rabies 2X RT-PCR Mastermix Or Control 2X RT-PCR Mastermix	10 µL
Nuclease-Free Water	10 µL
Total Volume	20 µL

Therefore, at a minimum, each PCR run will contain 6 separate RT-PCR reactions.

C. One-Step RT-PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one-step RT-PCR.

Table 4. Rabies Assay Program

One Step RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	Step 1	50°C	25 min
Cycle 2	Step 1	95°C	5 min
Cycle 3 (35x)	Step 1	94°C	15 sec
	Step 2	60°C	30 sec
	Step 3	72°C	45 sec
Cycle 4	Step 1	72°C	5 min
Cycle 5	Step 1	4°C	∞

D. Rabies One Step RT- PCR Assay Results Interpretation

1. For the analysis of the RT-PCR data, the entire 15-20 µL RT-PCR Reaction should be loaded on a 1X TAE 1.7% Agarose RNA gel along with 10 µL of Norgen's RNA Marker (provided). Prepare enough agarose gel for running one set of RT-PCR of Rabies detection and one set of RT-PCR for controls detection.
2. The RT-PCR products should be resolved on the 1X TAE 1.7% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

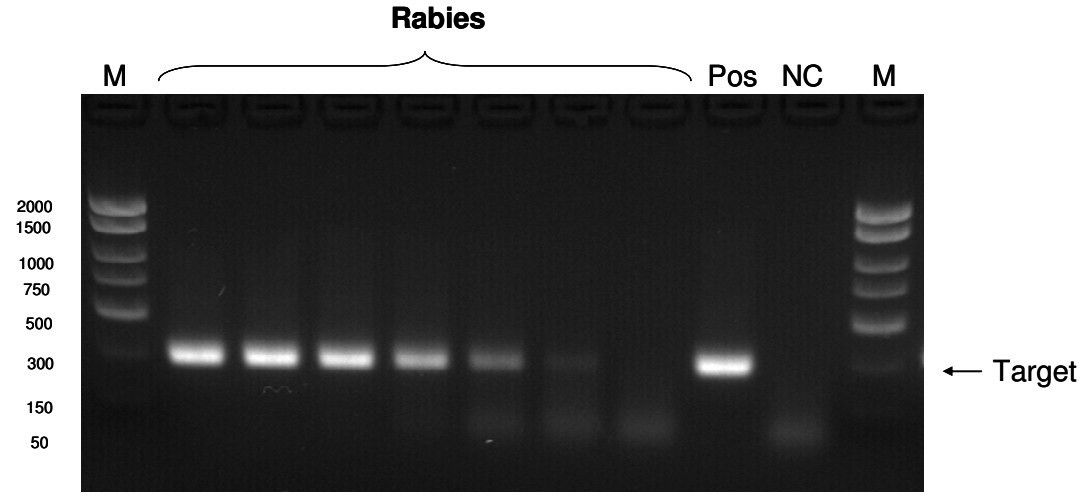

Figure 1: A representative 1.5X TAE 1.7% agarose gel showing the amplification of **Rabies** under different concentration (Target) using the **Rabies 2X RT-PCR Mastermix**. The size of the **Rabies** target amplicon corresponds to 280 bp as represented by the provided DNA Marker (M). **NC** = Negative Control.

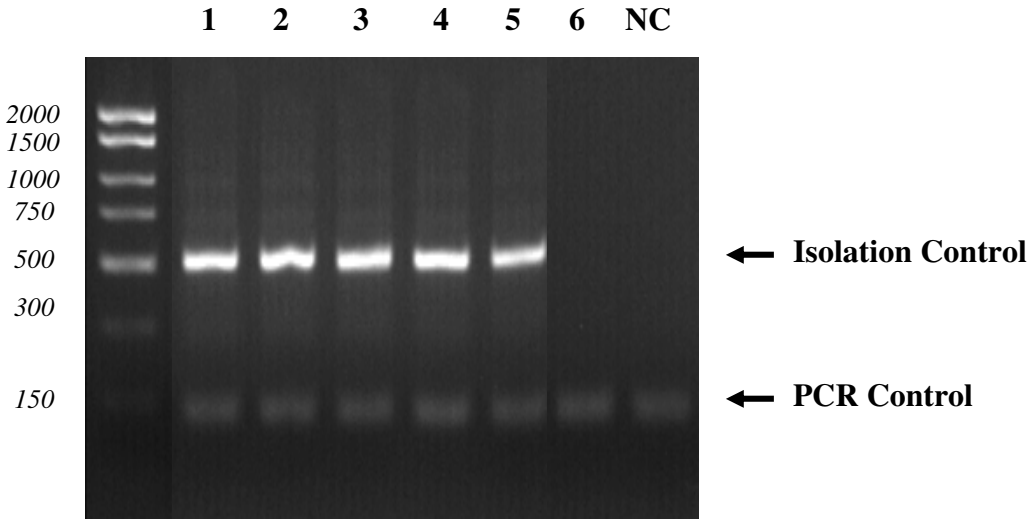

Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of **Isolation Control** and **PCR Control** under different conditions using the **Control 2X RT-PCR Mastermix**. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the RNA isolation as well as the RT-PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the RT-PCR was successful, the isolation failed to recover even the spiked-in Isolation control. **NC** = Negative Control.

Table 5. Interpretation of One-Step RT-PCR Assay Results

Input Type	Target reaction	Control Reaction		Interpretation
	Rabies Target Band (280 bp)	***IsoC* Band (499 bp)**	**Rabies *PCRC* Band (171 bp)**	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample			X	Re-test
Sample				Re-test
Sample		X		Negative
Sample	X		X	Positive
Sample	X	X		Positive
Sample	X			Re-test

** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

E. Rabies RT-PCR Assay Specificity and Sensitivity

- The specificity of Norgen's Rabies RT-PCR Detection Kit is first and foremost ensured by the selection of the Rabies-specific primers, as well as the selection of stringent reaction conditions. The Rabies specific primers were checked for possible homologies to all GenBank published sequences by sequence comparison analysis and published strains.

F. Linear Range

- The linear range of Norgen's Rabies RT-PCR Detection Kit was determined by analysing a dilution series of a Rabies quantification standards ranging from 100 ag to 1 pg.
- Each dilution has been tested in replicates (n = 4) using Norgen's Rabies RT-PCR Detection Kit on a 1X TAE 1.7% agarose gel.
- The linear range of Norgen's Rabies RT-PCR Detection Kit has been determined to cover concentrations from 100 ag to 1 ng
- Under the conditions of the Norgen's Rabies RNA Isolation procedure, Norgen's Rabies RT-PCR Detection Kit covers a linear range from 100 copies to 1 x 10^6 copies.

Frequently Asked Questions

1. How many samples should be included per RT-PCR run?

- Norgen's Rabies RT-PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

2. How can I interpret my results if neither the Rabies RT-PCR control nor the Isolation Control (*IsoC*) amplifies?

- If neither the Rabies PCR control nor the Rabies Isolation Control (*IsoC*) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify, therefore the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the Rabies PCR control showed amplification but neither the Rabies target nor the Isolation control amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the Isolation Control (*IsoC*) was amplified in a sample?

- The sample tested can be considered as Rabies negative.

5. How should it be interpreted if the Rabies PCR control and the Rabies target showed amplification in a sample?

- The sample tested can be considered positive. It could happen when too much template was added to the reaction.

6. How should it be interpreted if only the Rabies target and the Rabies PCR control were amplified in a sample?

- The sample tested can be considered as Rabies positive.

7. How should it be interpreted if only the Rabies target was amplified in a sample?

- It is recommended that the isolation is repeated.

8. How should it be interpreted if only the Rabies PCR control and the Rabies Isolation control showed amplification in a sample?

- The sample tested can be considered negative

9. What if I forgot to do a dry spin after my third wash?

- Your first RNA elution will be contaminated with the Wash Solution. This may dilute the RNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

10. What if I forgot to add the Isolation Control (*IsoC*) during the isolation?

- It is recommended that the isolation is repeated.

11. What if I forgot to run the Control RT-PCR for the sample and I only ran the Detection RT-PCR and I obtained a positive result?

- The result can be considered positive. However, any negative result must be verified by running the associated control RT-PCR to ensure that it is a true negative and not a false negative due to problems with the RNA isolation or the RT-PCR reactions.

Related Products	Product #
Total RNA Purification Kit	17200
Feline Leukemia Virus RT-PCR Detection Kit	44000
Feline Calicivirus RT-PCR Detection Kit	43900
Feline Herpes Virus PCR Detection Kit	44300
Feline *infectious peritonitis* RT-PCR Detection Kit	44400

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Rabies RT-PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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